DNA Analysis Lab

Pre-Lab Tasks:

1. Review the handout and **complete the tasks** as directed. For the task about cutting the DNA sequences, tape or paste them onto a separate paper and answer the related questions.

2. Review the procedures & analysis requirements.

I. Procedures

PART 1: Onion DNA Extraction

1. Add 2mL soap to the DNA tubes at your table, let sit for 5 minutes.

2. Put filter paper into a graduated cylinder and filter the DNA mixture for 15 minutes.

3. Clean out your test tube so that no soap or food sample remains in it.

4. Add the filtered DNA mixture (the liquid filtered out) to the tube.

5. Tilt the tube 45^{o} and slowly add 2mL alcohol to the tube and then raise the tube back vertically.

6. Use a wood stick to stir within the top alcohol layer and you should see white strings of DNA gathering on the wood stick.

7. Add the DNA to the petri dish and label with your name.

8. I will add a blue tracking dye to the DNA samples from your group after school and prepare the samples for analysis. The DNA samples will be combined for each group.

PART 2: Gel Electrophoresis DNA Separation

A. Preparing the Gel

1. Obtain a 200-250mL beaker and fill with DI water in the glass jars provided.
2. Add to a hot plate and heat until boiling.
3. To a 100mL graduated cylinder, add 63.7mL of this water.
4. Add the water to a 100mL beaker
5. Add 0.5g agarose powder & 1.3mL buffer; stir until completely dissolved.
6. Allow to sit for 10 minutes to cool down.
7. Add clear tape to the ends of the gel casting trays as per my demo.
8. Add the well molds to the casting trays in the slots as per my demo.
9. Add the liquid agarose into the casting tray until the molds' teeth are nearly covered.
10. Place trays into a plastic baggie and add to the green bin for storage until lab day.

B. Loading & Running the Gel

1. Add the tray to the chamber so that the colors match (red with red, black with black)
2. Gently pour the buffer solution provided into the chamber until the gel is completely covered.
3. Remove your DNA samples from the water bath & extract 1µL (or smallest amount possible)
4. Insert each DNA sample with a different pipette tip into:
    a. Left well – Insert the Onion DNA cut with HindIII
    b. Right well – Insert the Onion DNA cut with EcoRI
5. Wipe the inside of the chamber cover with a soapy paper towel (reduces fog)
6. Cover the chamber with the lid, connect to the power source.
7. Turn on the power source and adjust voltage to 125V.
8. Let run for 15-25 minutes, or until the blue tracking dye is close to the edge of the gel.
9. Turn off the power source, unplug and take off the cover.

PART 3: Staining the Samples

1. Carefully remove gel from chamber and add to a staining tray.
2. Fill a pipette with blue stain and add until the top of the gel is just covered but not spilling over.
3. Leave to stain for 5 minutes.
4. Carefully drain the stain off the top of the gel (drain into another staining tray).
5. Cover the gel slowly with water from a beaker (tap water is fine).
6. Agitate the tray gently to help stain leach out.
7. Repeat steps 5 & 6 until the water runs clear or the DNA bands are clearly visible.

II. Collection & Analysis of Results

For the analysis you will do **2 separate chi square calculations** for each restriction enzyme used.
The data below are the fragments' distances traveled (in mm) expected to result from being cut by each restriction enzyme. You must use this information & the provided standard curve (next page) to determine the expected values in Base Pairs.

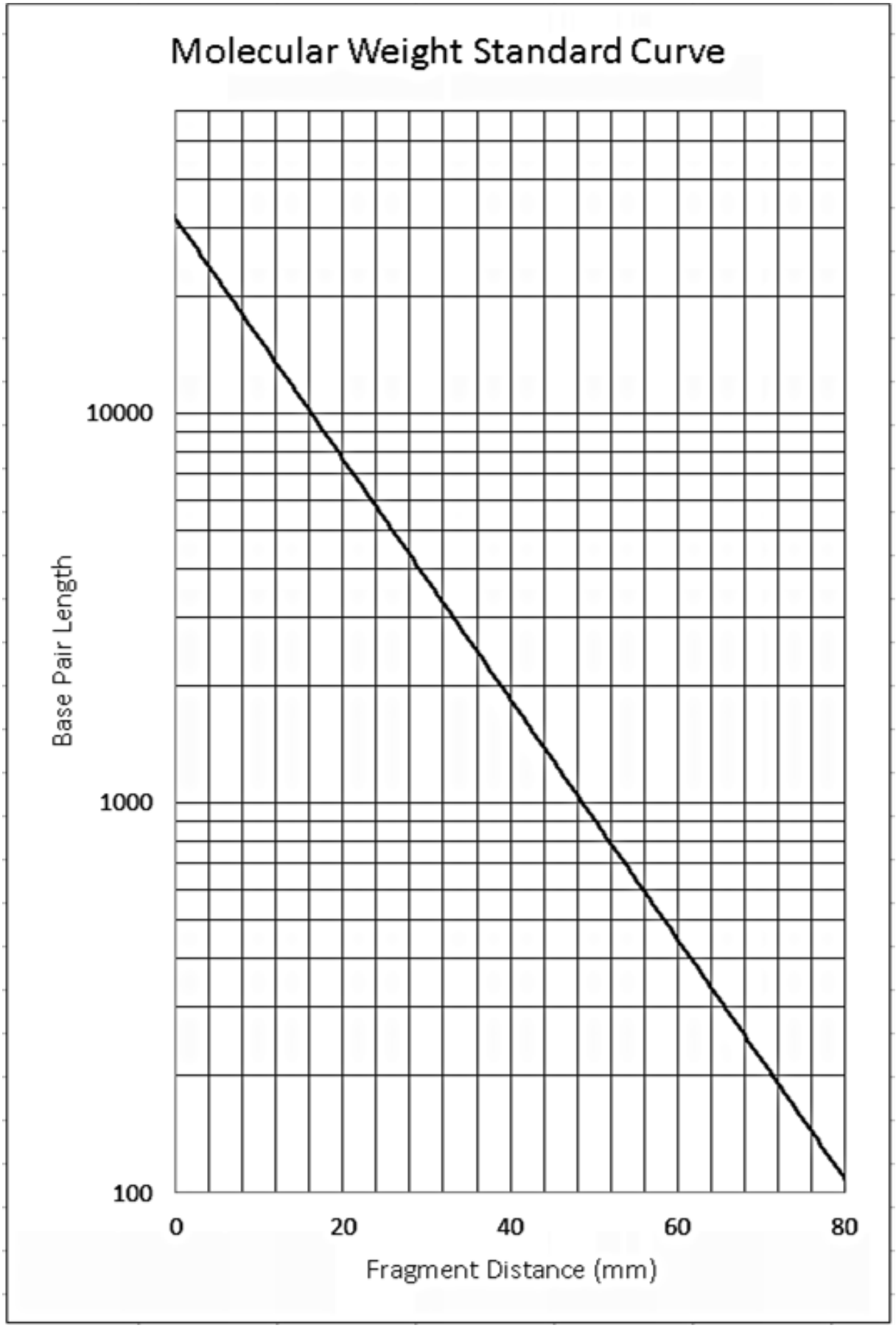
Figure 1: Standard distance traveled by each fragment resulting from each respective restriction enzyme.

	Fragment 1	Fragment 2	Fragment 3	Fragment 4	Fragment 5	Fragment 6
EcoRI	8mm	22mm	25mm	26mm	29mm	33mm
HindIII	6mm	16mm	21mm	27mm	38mm	39mm

For your group's results, use a metric ruler to measure the fragment distance traveled from the well face to the middle of each fragment. Record your data in a suitable table.

Convert these measurements into units of base pairs using the standard curve and use these values for the Chi square tests.

Conduct your chi square tests appropriately and perform all other lab report components as described in the general report guidelines handout.


Molecular Weight Standard Curve
10000
1000
100
Base Pair Length
0
20
40
60
80
Fragment Distance (mm)